MOLECULAR DYNAMIC SIMULATION OF MPPC INCORPORATED DPPC TO OBSERVE EFFECT ON PERMEABILITY

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MOLECULAR DYNAMIC SIMULATION OF MPPC INCORPORATED DPPC TO OBSERVE EFFECT ON PERMEABILITY

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SUMMARY

Liposome is a promising drug delivery system that can encapsulate drug inside whether it is hydrophilic or hydrophobic, avoid response of the body immune system, and safely deliver to the targeting tumor. The permeability of the liposome gets to the peak when it reaches the certain temperature, a gel to liquid crystalline phase transition temperature. However, due to the fact that the phase transition temperature is higher than the body temperature, it is difficult to control release of drug in the body. On the previous research, Needham et al. have shown that the phase transition temperature is lowered when liposome is incorporated with the lysolipid, thus enhancing the permeability. This study simulated MD simulation of lysolipid (MPPC) incorporated bilayer (DPPC), structured in two different configurations: dispersed and island. The 10% MPPC incorporated DPPC bilayer was simulated in two temperatures, 300K and 305K. The properties of the system were analyzed by interface formation energy, density profile, and pair correlation function.

CHAPTER 1

INTRODUCTION

Although there has been a development in the cancer treatment and medications, it is more important to have an effective method to deliver drug to the targeting tumor site. There are various types of drug delivery system: albumin conjugates, lectins, glycoproteins, DNA, dextran and liposome [1]. Among these, liposomes made of lipid bilayer is considered as promising drug delivery system.

Lipid bilayer is easily found in the living organisms in the cell to separate inner area from the outer area. It works as the boundary of the cell to control small molecules that move across the cells [2]. Phosphatidylcholine which makes up the lipid bilayer is composed of two different parts: choline head group and glycerophosphoric acid. When phosphatidylcholine is located in the aqueous solution, the hydrophilic head groups move toward solution and hydrophobic tails move toward inside and against the solution, thus forming lipid bilayer.

According to the Needham, when monopalmitoylphophatidylcholine (MPPC) is incorporated in the dipalmitoylphosphatidylcholine(DPPC), the lipid bilayer forms into the liposome for drug to be capsulated inside [3][4]. This liposome is thermos-sensitive vesicle that the permeability of the drug reaches peak when it reaches certain temperature [5]. Therefore, the release of the drug can be controlled. However, there is still limitations on study about structure of lysolipid, MPPC, DPPC, and about controlling the temperature that it reaches peak permeability.

This study will present molecular dynamic simulation of lysolipid incorporated lipid bilayer. 10% MPPC incorporated DPPC is prepared in two

different structures: dispersed and island. In dispersed structure, MPPC lipids are evenly distributed among the DPPC and, for island structure, MPPC lipids are clustered together like an island. We aim to analyze the how properties differ from the structure of liposome using the MD simulation.

CHAPTER 2

Literature Review

The biggest limitation with the current thermos-sensitive liposome is that the transition from gel to liquid crystalline phase is relatively higher than body temperature, 37°C, so it is difficult to reach the peak permeability in the body [6]. In 1999, Needham first proposed that the permeability at the phase transition from gel phase to liquid crystalline phase of liposome can be enhanced when it is incorporated with lysolipid by lowering the phase transition temperature [4][5]. 1-Palmitoyl-2-Hydroxy-*sn*-Glycero-3-Phosphocholine (MPPC) is incorporated into l,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC). While pure DPPC liposome release 20% at 40°C-45°C, 10% MPPC incorporation lowered transition temperature to 39°C- 40°C [7].

One of the reason that lysolipid enhances the permeability and lowers transition temperature of liposome is stabilization. When lysolipid is incorporated into the liposome, lysolipid stabilizes the pore, allowing tiny molecules to permeate through lipid bilayer easily. The previous study has proven this fact when liposome, encapsulating doxorubicin showed a release rate of 90% in 20 seconds [7]. In Northwestern University, a group of members studied permeability enhancement of lysolipid incorporated DPPC bilayer using coarse grained molecular dynamics. Liposomes with different concentration of MPPC were prepared and arsenic trioxide was encapsulated. They were simulated in different temperature like 25°C, 37°C, and 42°C to observe permeability of encapsulated arsenic trioxide [7]. It showed that MPPC incorporated DPPC bilayer shows a peak permeability in phase transition

temperature and 10% MPPC incorporation is most effective among concentrations. However, it needs a better understanding how stabilization of pore of the liposome increases the permeability of the system.

Another course grained molecular dynamic study by the same research lab has done simulation to observe permeability of the system in different temperature. For the Lateral diffusion coefficient against temperature, it showed that high concentration of MPPC does not show difference from pure DPPC or does not form gel. Moreover, the permeability coefficient was calculated to understand diffusion. To conclude, the enhanced permeability at the phase transition showed a sharp jump, indicating increase of the fluctuation in free volume [8]. Although, MPPC was well known for stabilization, MPPC with great concentration actually destabilized DPPC in the system and lowered the phase transition temperature.

All these studies have shown that MPPC incorporated DPPC lowers a phase transition temperature, thus increasing permeability of the liposome. However, none of these have shown in which structure they are formed. Moreover, these reports focused on varying concentration rather than varying temperature.

CHAPTER 3

Materials and Methods

To prepare lipid bilayer, MPPC, DPPC, and walter molecules are built using the cerius 2. The charge was given by Maestro. Simulation was done with the 10% MPPC incorporated DPPC in water phase with certain number of molecules as shown in Table 1.

	DPPC	MPPC	Water molecule
Number of molecules	108	12	6920

Table 1. Total number of molecules of DPPC, MPPC, and water molecule used in one system.

Both island and dispersed structure contain 120 lipids in 6920 water molecule in the form of lipid bilayer. To describe the lipid bilayer, those lipids were distributed into two layer: top and bottom. Dreiding force field by Mayo was given to the MPPC incorporated DPPC. In the force field, the potential energy includes torsion energy, angle, bond, electrostatic and van der Waals. All the simulations were done in LAMMPS. The charge was given to the molecules using Jaguar and lipid bilayer was heated from 15K to 300K in 250 ps. Then NVT MD simulation has been done for lipid bilayer. Lipid bilayer went through energy minimization with cerius 2 and energy minimization for water molecules have been carried out with LAMMPS with basic set of 6-31G** and B3LYP. It was done with the Mulliken Population analysis in water solvent and NPT ensemble with 1atm. The water molecules were prepared with the NVT MD simulation at 300K. The water phase has been combined with the lipid bilayer, energy minimization was carried and NVT and NPT MD simulations

has been done again for entire system. NVT was done in 400 ps at 300K and NPT was done in 5 ns at 300K.

CHAPTER 4

Results and Discussion

Interfacial Formation Energy

Interface formation energy is measure interaction of lipid during the formation of lipid bilayer in aqueous solution. IFE was calculated to compare stability between "dispersed" and "island" structure. It is calculated with the equation below.

$$IFE = \frac{(E_{total} - (n \times E_{lipid,single} + E_{water}))}{n}$$

In the equation, n is number of lipid in the system, E_{total} is energy of system, $E_{lipid,single}$ is energy of a single lipid and E_{water} is energy of water molecule in the system.

Energy [kcal/mol]					
	E_{total}		$E_{lipid,single}$		E_{water}
	Dispersed	Island	DPPC	MPPC	
300K	-65600±182	-65700±173	172±8.53	120±7.24	-69400±152
305K	-64800±176	-64800±184	169±11.0	124±8.36	-68800±146

Interface Formation Energy [kcal/mol]		
	Dispersed	Island
300K	-135±8.14	-131±10.5
305K	-136±8.22	-131±10.4

Table2. Interaface formation energy of both systems in 300K and 305K

Results in the Table 2, shows that dispersed system has lower interface formation energy, but it falls into a standard deviation. Therefore, it indicates that there is no statistically significant difference between interface formation energies.

Pair Correlation Function

Radial distribution function (RDF) is one of the useful analysis show overall structure and interaction between atoms of the molecules in the system. Moreover, the distribution and correlation can be understood with the result. Pair correlation function is simply understanding the probability of finding certain atom at a distance r from another atom.

$$g_{A-B}(r) = (\frac{n_B}{4\pi^2 \Delta r})/(\frac{N_B}{V})$$

n_b is number of atoms B located at the distance r with the thickness of Δr from atom A, N_B is number of B atoms in system, and V is the total volume.

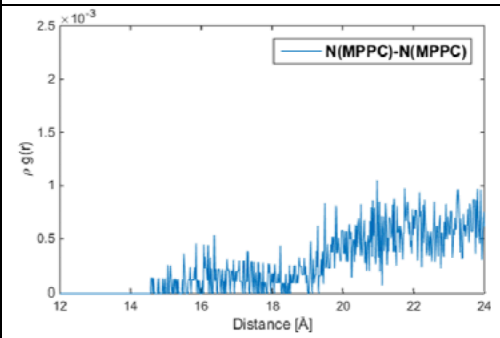
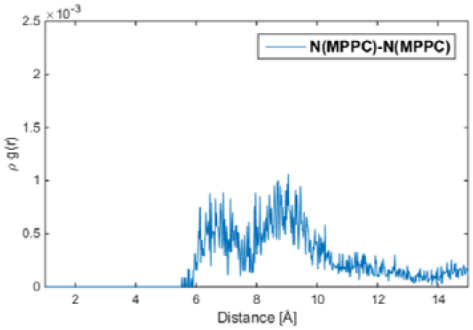
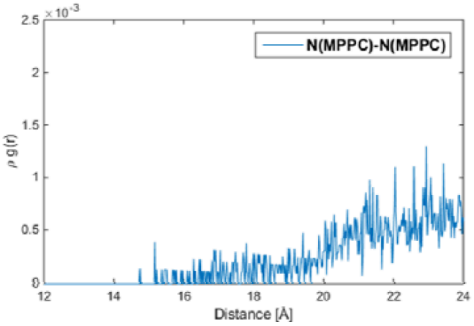
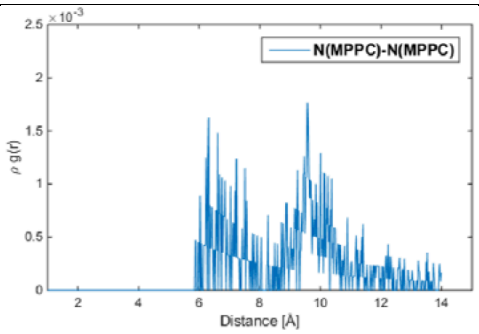
	Dispersed	Island
300K		
305K		

Figure 1: Radial distribution function of nitrogen-nitrogen MPPC in 300K and 305K for island and dispersed configurations

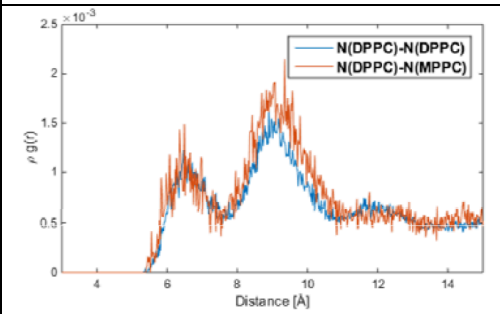
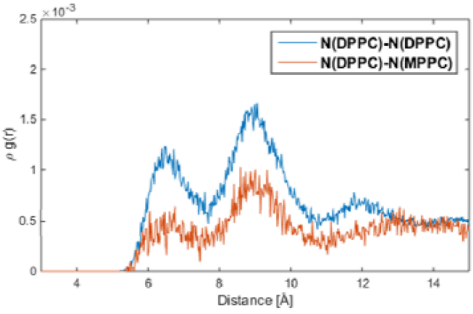
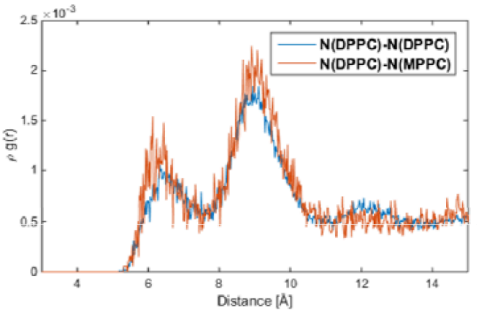
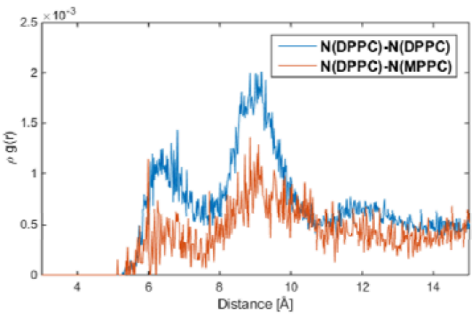
	Dispersed	Island
300K		
305K		

Figure 2: Radial distribution function of nitrogen of DPPC with nitrogen of DPPC or MPPC in 300K and 305K for island and dispersed configurations

Figure 1 and 2 shows the distribution of MPPC in island and dispersed system. In Figure 1, the graph shows that while the distance between nitrogen of MPPC are at least 14.5 Å in dispersed structure and the island structure shows that MPPC are more closely located. The minimum distance between MPPCs decrease to 6 Å. In figure 2, N(DPPC) to N(MPPC) of island structures shows lower intensity than those of dispersed structure. It is because in the island structure, MPPCs are clustered together forming island, while they are distributed among the DPPCs in the dispersed structure.

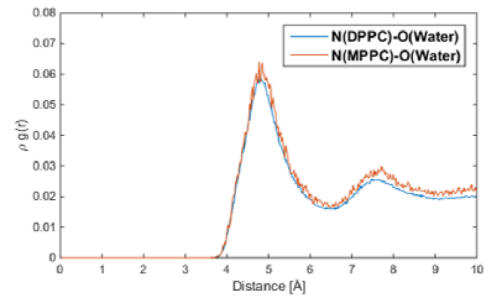
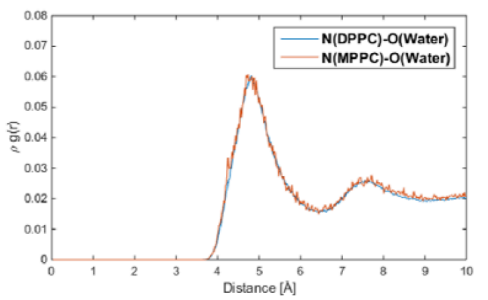
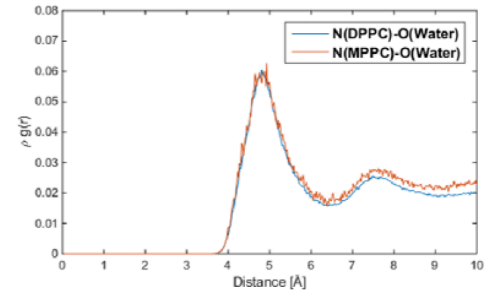
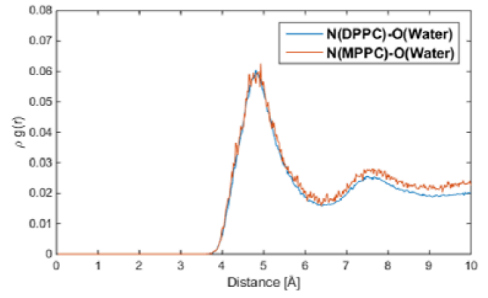
	Dispersed	**Island**
300K		
305K		

Figure 3: Radial distribution function of nitrogen of DPPC and MPPC with oxygen of water in 300K and 305K for island and dispersed configurations

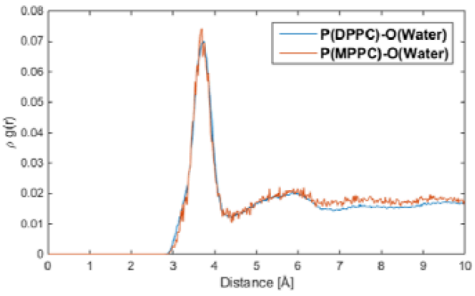
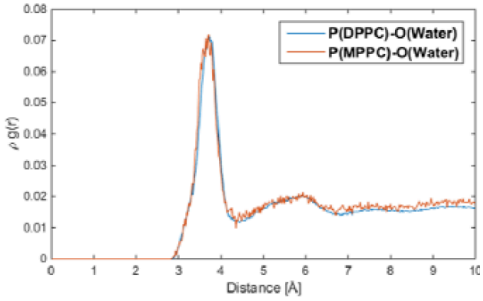
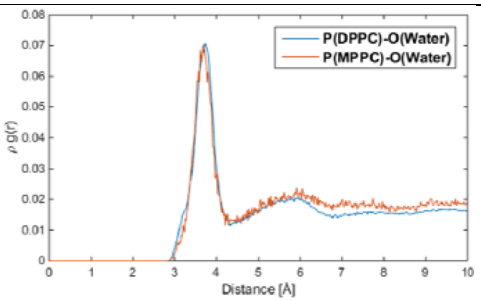
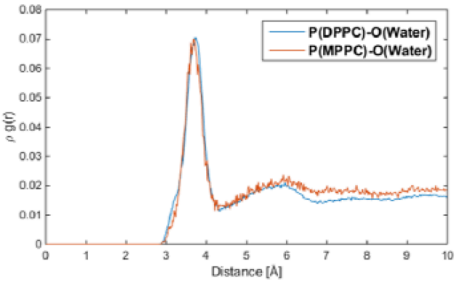
	Dispersed	Island
300K		
305K		

Figure 4: Radial distribution function of phosphorus of DPPC and MPPC with oxygen of water in 300K and 305K for island and dispersed configurations

Figure 3 and 4 shows distribution of water and head group for both systems in 300K and 305K. N(DPPC) to O(water) shows similarity between systems and peak. However, the intensity of N(MPPC) and O(water) of dispersed system is higher than N(DPPC) to O(water). However, there is no significant difference observed.

Density Profile

Density of molecules of the system are calculated across z-axis. It shows a density of MPPC, DPPC, water molecules, and atoms of lipids. Figure 6 shows that the density of water is high at both end of the graph, it is because the lipid bilayer is located in the aqueous solution and the water does not permeate all the way to the center. For DPPC, there is a dip in the 0 of z coordinate, because there is a space

between DPPCs for tails. Comparing to dispersed configuration, island system has higher density of DPPC and MPPC tail, because they are packed more tightly than those of dispersed system.

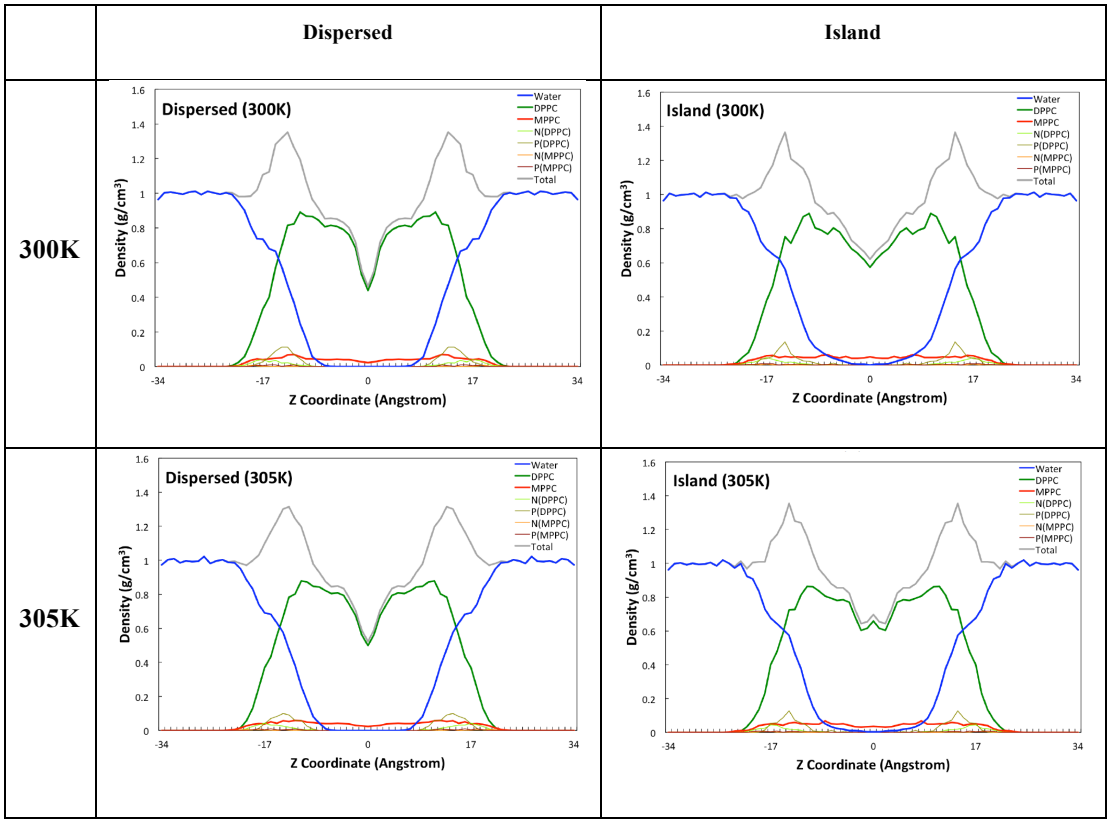


Figure 6. Density profile along the z-axis for both systems in 300K and 305K

Chapter 5

Conclusion and Future Works

Based on the results of simulations of both systems in 300K and 305K, MPPC incorporated DPPC showed that water permeates through the lipid bilayer. Moreover, the island system is shown as more stable system than dispersed system. The density of atoms and RDF was resulted to be slightly different between systems. However, the amount of data is not enough to draw a single conclusion. Therefore, for future work, the simulation has to be done with more various temperatures such as 310K, 315K, and 320K which are around the phase transition temperature. Software for MD simulation can be switched to the gromacs from LAMMPS, because gromacs is more applicable and fast in simulation time. Moreover, other than RDF, density profile, and interface formation energy, more data analysis such as mean square displacement can be calculated.

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